VII.4 An Attempt at Classical Biological Control of Rangeland Grasshoppers With *Entomophaga grylli,* Pathotype 3

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The primary objective of this project, conceived and developed by R. I. Carruthers, was to develop and implement a classical biological control program against rangeland grasshoppers using an Australian isolate (pathotype 3) of the grasshopper obligate *Entomophaga grylli* (Zygomycetes: Entomophthorales) species complex (Ramoska et al. 1988). Pathotype 3 was isolated from *Praxibulus* sp. grasshoppers in Australia in 1985 by R. S. Soper and R. J. Milner during an epizootic (grasshopper outbreak) (Milner 1985).

The project was based on the collaborative findings of Soper and Milner and a 5-year study of the two native species designated pathotype 1 and 2 in Arizona and New Mexico (Carruthers and Humber 1988 unpubl.).

Implementation of the attempt (Carruthers and Humber 1988 unpubl.) was through the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA, APHIS, PPQ), Grasshopper Integrated Pest Management (GHIPM) Project initiated in 1987.

The proposed and pursued approach was the introduction, establishment, evaluation, and dispersal of an Australian isolate, pathotype 3, to augment the two native pathotypes (1 and 2). Previous laboratory studies indicated that pathotype 3 had a wider host range than either of the native species plus other attributes that led to its selection (Ramoska et al. 1988).

These fungi, along with other biotic agents could theoretically provide long-term, nonchemical suppression of grasshoppers between outbreaks.

An ecological survey of sites with histories of grasshopper populations and densities suitable for introduction was made within the 17 Western States. The Little Missouri National Grasslands in McKenzie County, ND, was selected as the initial study area for field evaluation of pathotype 3 (Carruthers et al. 1989 unpubl.).

The use of biological control methods for grasshopper management, and specifically the introduction of the Australian fungus, was supported by the membership of the McKenzie County Grazing Association, Watford City, ND.

Administrative policies and technical procedures within Federal agencies and the State of North Dakota in effect at the time were observed and provided guidance for introduction. Permission was granted for field studies in North Dakota (Carruthers et al. 1989 unpubl., and in press).

The goals of proposed releases were to reduce populations of economically important grasshoppers on western rangeland to, or below, threshold densities; to establish pathotype 3 as a biorational agent that would augment native fungi; and to determine the plausibility of future large scale releases throughout the Western United States by PPQ's Plant Protection Laboratories.

Pathotype 3 was introduced into susceptible grasshopper populations at several sites in McKenzie County in 1989, 1990, and 1991 and at two sites near Delta Junction, AK, in 1990 (Carruthers et al. 1989 unpubl., 1990 unpubl., 1991 unpubl.).

Introduction was by randomly releasing laboratory-infected fifth-instar and adult *M. differentialis* (Thomas), each injected with 10 µl of 10^4 pathotype 3 protoplasts, into grasshopper populations in alfalfa/mixed grass or crested wheatgrass fields with no history of pathotype 1 or 2 fungus infection. Each field was about 44.5 acres (18 ha) in size. Releases were made at 2- to 3-day intervals 3 days postinjection (just prior to death of the grasshopper). Weekly releases in lots of 500 infected grasshoppers totalled from 500 to 3,500 at each site.

The initial release of pathotype 3 was made July 24, 1989. Five hundred (500) infected grasshoppers were released in an alfalfa/mixed-grass hayfield at Wold's ranch (T153N, R97W, Sec. 33), 25 miles north of Watford City, ND. Incidence of fungus infection among grasshoppers within this release site was 13 percent 2 weeks after the release (Carruthers et al. 1989 unpubl.).

Additional releases of ca. 500 per day were made at Wold's on July 8, 11, 15, 19, 25, and 30, 1990. A 20-percent incidence of infection was observed at this site within 2 weeks of the 1990 releases. No additional releases were made at this site after 1990.

Similar releases were made in crested wheatgrass fields at three other sites in McKenzie County, ND, during 1990. Incidence of fungus infection among grasshoppers at these locations was less than 3 percent. Low incidence of infection in these fields was attributed to the open canopy of the crested wheatgrass, which likely resulted in a less favorable habitat for the fungus (Carruthers et al. 1990 unpubl.). Seasonal monitoring of grasshopper populations at these sites (1991–94) has failed to detect fungus-infected individuals.

The releases of pathotype 3 into *M. sanguinipes* populations at two sites in Alaska were considered unsuccessful in that only a single sporulating cadaver was recovered 2 weeks after release. Grasshopper populations at these release sites have been monitored annually for incidence of fungal infection.

Overwintering of pathotype 3 was thought to occur in Wold's field based on recovery of sporulating *M. bivittatus* (Say) cadavers in June, 1991. Fungal mortality among grasshoppers at this site reached 26 percent in 1991 even though no additional introductions were made (Carruthers et al. 1991 unpubl.).

Releases of infected grasshoppers (500–1,000 each) were made on land managed by the U.S. Army Corps of Engineers near Lake Sakakawea (T154N, R95W, Sec. 32) on June 6, 8, 11, and 13, 1991. The incidence of fungus infection at this location reached 25 percent 2 weeks after the last release. No additional releases were made after June 13, 1991. Grasshopper populations at this site continued to be monitored for incidence of fungal disease through 1994. Populations and incidence of fungal infection have been diminishing since 1991.

The initial success in North Dakota was encouraging, and a plan for additional releases of 150,000 infected *M. differentialis* (10,000 per week per location for 5 consecutive weeks) at 3 other locations was drafted. Additional releases were contingent upon production and supply of suitable hosts by a commercial insectary in Colorado. The number of sites and infected grasshoppers to be released was based on available human and fiscal resources as well as host population densities.

This project and plans for future releases of nonnative pathogens and parasites within the GHIPM Project caused intense debate among certain researchers and between agency administrators in 1991 (Bomar and Lockwood 1991, Lockwood 1993a and b, Carruthers and Onsager 1993). In August 1991, amid the beginning controversy of the legality and wisdom of this approach, the principal investigator (R. I. Carruthers) was reassigned, and the project was transferred from Ithaca, NY, to me at Kimberly, ID.

Additional documentation was drafted and submitted (April 1992) and revised and resubmitted (October 1992) seeking a policy decision on the need for an environmental assessment (EA) before proceeding with additional releases of pathotype 3.

Additional releases of pathotype 3 are stalemated. No releases of pathotype 3 have been made since June 1991. Efforts since that time have been relegated to monitoring (population densities, composition, species fluctuations, incidence of mortality due to fungus infection, dispersal studies) in the release field and surrounding areas.

Laboratory studies were conducted to establish basic parameters of conidia production, germination and viability, and dose/mortality curves, as well as mass inoculation methods that would be required if the project was to be assumed and enlarged by PPQ's Plant Protection Laboratories.

The development of DNA probe technology for separation and identification of three *Entomophaga* spp. of the *E. grylli* complex has also been successful. Cooperation between USDA's Agricultural Research Service staff scientists at Ithaca, NY, and Kimberly, ID; the Boyce Thompson Institute for Plant Science; and the University of Toronto, Scarborough campus led to the development of a positive DNA identification probe whereby pathotypes 1, 2, and 3 can be separated and positively identified (Bidochka et al. 1995). This is a critical accomplishment and provides a tool necessary to delineate dispersal and distribution of pathotype 3 in the field.

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